Summary of Doctoral Thesis

Enrollment year: 2019y   10m
UGAS Specialty: Plant Production
Name: RUMAN MD HAFIJUR

Title	**Characterization of lysin motif receptor-like kinases in plant**

Plant survival in the rhizosphere depends upon the recognition and interaction with soil microorganisms, especially bacteria and fungi, which are abundant in the soil. The interaction between plants and microbes is not always detrimental but sometimes benefits both partners. The ancient and widespread beneficial symbiosis is the arbuscular mycorrhiza symbiosis (AMS), based on mineral nutrients and fixed carbon exchange between mycorrhizal fungi and more than 80% of plants on the land. Another agronomically crucial root nodule symbiosis (RNS) occurs between legumes with Rhizobia supplying nitrogen to plants in exchange for carbohydrates. The rest of the soil microbes are pathogenic and infect the host for survival. However, plants evolved their defense mechanism to tackle the invaders and, at the same time, recruit beneficial symbionts into the root. In the case of pathogenic interaction, plants recognize molecular compositions of invading microbial constituents called microbe-associated molecular patterns (MAMPs). MAMPs perception by plants through pattern recognition receptors (PRRs) activates the immune system. On the other hand, plants can recognize molecular cues synthesized by beneficial microorganisms through specific receptors and establish symbiotic relationships. Pathogenic and beneficial, both types of microbes secrete molecular compounds such as short and long chitooligomers, lipochitooligomers, peptidoglycans with N-acetyl glucosamine as the major component, and β-glucans, exopolysaccharides, and lipopolysaccharides are recognized by lysin motif (LysM) receptor-like kinases (LysM-RLKs), plant-specific receptors mainly localized at the plasma membrane and activating either innate immunity or beneficial symbiosis.

LysM-RLK comprises two functional regions across the transmembrane domain: an extracellular region carries one to three LysM domains, and an intracellular kinase region contains a kinase domain. LysM-RLKs can be categorized as active and pseudokinases based on kinase domain catalytic activity. From ancient to modern plant species have LysM-RLKs and many are characterized as Nod factors, Myc factors, chitin, peptidoglycan, and exopolysaccharide perceived and signalling to down-stream. Despite these receptor-like kinases' pivotal role in plant interaction, emerging presence, and eccentric structural formation, most receptor functions are yet to be identified. Additionally, the functionality prediction of newly identified LysM-RLKs is still puzzling. The previous study classified LysM-RLKs into three major groups (LYS-I, LYS-II, and LYS-II) based on the molecular phylogeny of the kinase region (Arrighi et al., 2006; Lohmann et al., 2010); however, estimating the function of each

plant's LysM-RLKs, their interaction with various microorganisms, and evolutionary events from this classification are challenging to evaluate. Therefore, in this chapter, I tried to combine previously reported characterized and uncharacterized LysM-RLK family and mined 20 seed plants genome databases to search for additional LysM receptors to report a comprehensive phylogenic characterization analysis. I extracted 215 LysM-RLKs from 20 plant species, including L. japonicus, and proposed a new classification of 16 clades fused into LYS-I, LYS-II, and LYS-III groups. My findings predict LysM-RLKs appearance and disappearance during evolution and estimate novel receptor functions in the interaction of soil microorganisms in Spermatophytes.

Therefore, in chapter 1, I updated information of LysM-RLKs in *Lotus japonicus* and characterized these 20 LysM-RLKs. To identify LysM-RLKs from diverse plant species and constructed a phylogenetic classification by using full-length amino acid to address the evolutionary and functional characteristics, the phylogeny analysis supported by protein homology and kinase functionality categorized 193 LysM-RLKs from 16 angiosperm species into 14 distinct clades and one out-clade receptor. Classification revealed that selected species possessed clade I receptors, mostly recognized as Nod factors/chitin receptors, and clade III receptors which are uncharacterized but conserved from ancient Bryophytes expect Physcomitrella patens. This study also identified dicots and monocots-specific clades and predicted the evolutionary drift of LysM-RLKs. Also, a correlation was found between the species that lost AMS and the lack of clade II receptors. To understand the evolutionary pattern and divergence of LysM-RLKs in different plant species, a phylogenetic lineage analysis of selected angiosperm orders and linked it with ancient green algae, bryophytes, and vascular species to explain the appearance and disappearance clades of LysM-RLKs was constructed. At first, non-land species green algae species *Chara braunii*, two bryophytic species, *Marchantia polymorpha, P. patens*, and one vascular species *Selaginella moellendorfii* genome databases were analyzed to extract LysM-RLKs. A NJ-phylogenetic tree was prepared including 22 LysM-RLKs of ancient species with 193 LysM-RLKs from 16 angiosperms. Combined phylogenetic analysis by using full-length, extracellular region, and kinase regions showed that *M. polymorpha, P. patens* and *S. moellendorfii* 9 LysM-RLKs were distributed as sub-clades within previously designated 14 clades named clade I' and III', and 5 LysM-RLKs were classified as new clade, *C. braunii* 7 LysM-RLKs were clustered as an independent clade This classification data also indicate that ancient species possessed 1 to 4 clades of receptors far less number than modern plants.

The phylogenetic classification, clade characterization, and evolution of LysM-RLKs allowed us to predict their evolutionary relationships and to infer new functions of uncharacterized receptors. In future, this clade classification can be used to elucidate the mechanisms of signalling interaction between LysM-RLKs and soil microorganisms.

L. japonicus Gifu genome comprised of 20 LysM-RLKs among them NFR1 to LYS8 are predicted active kinases and NFR5 to LYS18 are predicted pseudokinases (Chapter 1). Of these, NFR1/NFR5 were recognized Nod factors and LCOs perception receptor (Madsen et al., 2003; Radutoiu et al., 2003). LjNFR5 paralog LYS11 was also reported to be an AM-induced receptor (Rasmussen et al., 2016). The previous expressional and mutant study on EPR3 receptor has clearly demonstrated *Epr3* gene functions as root nodule infection thread formation and assessed the compatibility of exopolysaccharide secreted by *Rhizobia* bacteria (Kawaharada et al., 2015, 2017).

In chapter 1, I showed that, non-mycorrhiza Angiosperm species lost clade II and IV receptors. In AM associated plants, clade IV receptors including OsLYK5/NFR5, SlLYK10 regulated AM symbiosis, and these receptors with LjLYS11 were expressed during AM symbiosis (Buendia et al., 2016; Miyata et al., 2016; Rasmussen et al., 2016). Previous analysis also suggested that non-mycorrhized species simultaneously lost LysM-RLK genes which are regulated AM symbiosis. On the other hand, the function of the clade II receptors in AM associated plants have not yet understood, even though receptors were lost in non-mycorrhized plants. *L. japonicus* two receptors EPR3 and LYS8 are in clade II and therefore, in this chapter 2, I focused on the function of *L. japonicus* LysM-RLKs two receptors during root nodule and arbuscular mycorrhiza symbiosis. To identify the functions of these LysM-RLKs, I have performed expression and mutant screening of Epr3 and Lys8 genes.

Expression of the *Epr3* promoter region was highly induced in root nodules and nodule primordia whereas, *Lys8* gene showed expression in the arbuscule-containing cortical cells and vascular regions. Mutant analysis of *epr3* and *lys8* showed that mutation of *Epr3* and *Lys8* genes has an inadequate effect on root nodule and arbuscule formation, suggesting two receptors have any redundant roles in the *Lotus-Rhizobia* and Lotus-AM fungi symbiosis. The analysis suggests that both EPR3 and LYS8 are not essential and have no role during pre-symbiotic signalling however, EPR3 acts during RNS and LYS8 positively regulates AMS.

EPR3 and LYS8 are kinase-active receptor kinases, which can be autophosphorylated and transmit the signal to downstream components to regulate root development. Another LysM-active kinase receptor in rice, CERK1, a recognized symbiotic and immunity receptor together with OsNFR5 and OsCEBiP operate in lateral root formation and root development (Chiu et al., 2018). GUS staining analysis using transgenic plants carrying *Lys8prom::GUS* indicated that *Lys8* expression could be strongly detected in the vascular regions of whole roots in both inoculated and uninoculated transgenic roots. While *Epr3* promoter has no such expression in any of the root developmental organ except nodules. The uninterrupted expression pattern of the *Lys8* gene in the vascular regions suggested that this gene might involve in root growth and development independent of AMS.

The expression of *Epr3* and *Lys8* promoter region showed different expression patterns as *Epr3*

gene expressed in root nodules during symbiosis with Rhizobia such as *M. loti* whereas, *Lys8* is not specific to AM fungi *R. irregularis* because the expression of *Lys8* promoter region was observed in the transgenic root vascular region without any influence of microorganisms. Previous studies of receptor gene families showed that promoter regions are the primary sites for evolutionary divergence (Nimchuk et al., 2015). This study shows that, EPR3 and LYS8 can perform symbiotic molecular function in root nodules and arbuscular cortical cells expression site, however EPR3 might neo-functionalized.

Considering the diversity of LysM-RLKs in this thesis work, it can be concluded that plants are still carrying this diverse group of receptor protein to survive and to be benefited at the same time. LysM-RLKs are multifunctional type receptor like kinases which evaluated and helps plant to interact with variety of microorganisms. The classification of LysM-RLKs also presented an evolutionary origin and diversification from ancient to modern plant species. Also, a new clade of receptors is identified which functioned in root endosymbiosis. The discoveries and prediction from this work will help more to understand the complex nature of LysM-RLKs in future.

Note 1 The summary will be published online. Please confirm that UGAS has your permission to do so.

Note 2 In the summary, please do not include any content beyond your control (e.g. patents or intellectual assets) which will make online publication impossible.

Note 3 The summary should be four or five pages in total.